REMARKS

Claims 1, 2, 4 and 7-10 are pending in this application. By the present Communication, no claims have been added, canceled or amended. Applicants have considered the remarks set out in the Advisory Action mailed November 7, 2005, and respectfully traverse all grounds of rejections for the reasons that follow.

Rejections Under 35 U.S.C. § 103

Claims 1, 2, 4 and 7-10 stand rejected under 35 U.S.C. § 103(a) as obvious over Edwards et al. (2000) in view of Varner et al. and Berry. In maintaining this ground of rejection, the Office attempts to distinguish Applicants argument that the combination of Edwards et al., Varner et al. and Barry fail to teach or suggest re-computing optimal properties of an *in silico* biochemical reaction network until a desired optimal function is reached and cultivating a genetic makeup of a cell having the resultant reaction network to evolve to the previously determined optimal function when the reaction network is replicated in a cell. Applicants will address below each of the apparent distinctions alleged by the Office.

The Office alleges that Applicants previously argued that Edwards et al. does not teach flux balance analysis (FBA). Applicants respectfully point out that this argument was made in connection with the Berry reference, not in connection with Edwards et al.

In this regard, the Office has alleged that Berry describes an *in vitro* method, including flux analysis, for optimizing production of products by culturing genetically engineered cells under conditions that allow the cells to evolve to a desired or enhanced level of production. Applicants responded, in part, stating:

> Barry describes fed-batch fermentation and biosynthetic pathway manipulation unrelated to evolution in culture and concludes that implementation of a genetic alteration redirecting carbon availability may be promising. Further, Berry's use of the term "flux analysis" refers to the technique of experimentally determining the flux distribution of metabolism. This use is distinct from the Applicants use

> of "flux balance analysis" in the claimed invention because flux balance analysis refers to the technique of calculating an optimal flux distributions computationally.

Applicants' response filed October 14, 2005, at page 7, first paragraph (emphasis added; citations omitted).

Hence, Applicants previous response pointed out that any method purported to be described by Berry relates to fed-batch fermentation and that Berry's "flux analysis" corresponds to an experimental measurement and not a computational determination.

In the current Action, the Office alleges that Berry describes cultivating cells under conditions that allow cells to evolve to a desired optimal function allegedly because "under conditions of IPTG production, cells 'evolve' to produce DAHP." Advisory Action, *citing* Figure 3 at page 253 in support.

Applicants respectfully point out that IPTG is an inducer of the lac promoter and that the DAHP gene was placed under the control of this promoter so that expression could be induced by IPTG. In particular, Berry describes that the *aro*G gene, encoding the first enzyme of the DAHP synthase pathway was cloned under the transcriptional control of tandem lacUV5 promoters (see, for example, page 251, last paragraph). Similarly, Figure 3's legend expressly states that IPTG was added to "induce expression of *aro*G^{fbr}." *Id.* at page 253.

As an inducer of DAHP expression under the control of a lac promoter, IPTG turns on transcription of the DAHP gene (e.g., *aro*G^{fbr}). Therefore, Berry turns on expression of a gene product by the addition of a specific regulatory molecule. Turning on expression of a targeted gene by IPTG addition is not culturing cells to evolve as is claimed by the invention because it is a specific molecular induction gene transcription.

In further support of the contention that IPTG induced gene expression allegedly constitutes culturing cells to evolve to a desired optimal function, the Office appears to allege that Figure 3 shows a selective response to a change in the environment because some of the

IPTG induced cells in Figure 3 change or evolve to Berry's desired function whereas some of the cells fail to change or change in a negative direction. Applicants respectfully point out that such an interpretation is inconsistent with the results and discussion relating to Figure 3 in Berry.

Figure 3 shows the production of DAHP in *E. coli* strains that have different genetic constructs introduced for the purpose of improving the availability of aromatic pathway precursors D-erythros 4-phosphate (E4P) and phosphoenolpyruvate (PEP) (see, for example, page 252, col. 2, para. 2-3, and Figure 3 legend). The *E. coli* host strains for each set tested are isogenic, indicating that the genetic background of the host is identical. Further, there is no indication that growth or any other environmental condition was changed between tested isogenic constructs or during the course of induction. Therefore, the differences between the strains shown in Figure 3 are unlikely to be due to environmental selection. Rather, the observed differences between isogenic strains are likely the result of the different genes that have been introduced and induced. Berry confirms as much when Berry describes:

> Amplification of the tktA gene (encoding transketolase) was shown to increase the availability of E4P for aromatic biosynthesis (Refs 14;15; Fig. 3). Compared with a control strain that had wild-type levels of transketolase, the strain with amplified levels of transketolase produced DAHP at a higher rate and to a 3.6-fold higher final concentration (solid triangles versus solid circles, Fig. 3).

Id. at p.252, col. 2, para. 4 through page 254, col. 1, para.1 (emphasis added).

According to Berry, the difference observed between control and modified isogenic strains is due to the induced expression of the heterologously introduced gene. Therefore, the outputs for the strains in Figure 3 are different because the strains are genetically different and not because of any environmental change or selective response. Accordingly, Berry does not describe cultivating cells under conditions which allow cells to evolve to a desired optimal function because Berry specifically induces heterologous gene expression with IPTG and such differences are due to the overexpression of the induced gene rather than any environmental or selective response.

The Office also alleges that the cells of Berry have been metabolically engineered and, combined with the assertions above, satisfy the elements of steps (e) and (f) of claim 1. Applicant has addressed the assertions above. With respect to the Office's contention that the metabolically engineered cells constitute the cells is steps (e) and (f), Applicants respectfully point out that, by reference to step (d), steps (e) and (f) claim placing a genetic makeup of a cell constructed to contain biochemical reactions identified by the repetition of the *in silico* process recited in steps (a) - (c). There is no mention in Berry of an *in silico* process nor of repeating such and *in silico* to until a desired optimal function is reached. Therefore, and in contrast to the assertions by the Office, Berry, alone, does not describe all the elements of steps (e) and (f) of claim 1.

Edwards et al. has been cited by the Office allegedly because Edwards et al. describe a method of determining optimal growth in *E. coli* using a computational metabolic flux balance analysis. The Office previously conceded that Edwards et al. does not describe culturing engineered cells to allow expression of an optimal function but alleges in the Advisory Action that Edwards et al. describes experimental confirmation of his *in silico* prediction.

Applicants respectfully point out that the issue is not whether Edwards et al. or the secondary references describe or suggest culturing of engineered cells for the production of a desired product as it appears to be framed by the Office. Rather, the claimed invention is directed to creating an *in silico* model through a repeated process until a desired optimal function is reached in the model and then culturing a genetic makeup of that model for a sufficient period to allow cells to evolve to the desired optimal function. As taught in the application, cultivating cells to evolve to the desired optimal function identified by the claimed *in silico* steps is a process involving more than just culturing. In this respect, the application teaches:

> Using the optimization procedure, the properties of the corresponding actual biochemical reaction network may not be optimal or the same as desired from a practical standpoint. The simulated reconstructed network and its synthesis in an organism may not display the optimal solution desired, also referred to herein as the desired optimal performance or desired optimal function.

Application at para. [0061], and

> The resulting engineered cell may or may not display the optimal properties calculated ahead of time by the *in silico* methods using the iterative optimization procedure described above. . . . After the cell has been constructed to have a potential to meet the desired performance it is placed in culture under a specified environment. The specified environment is determined during the optimization procedure. That is, the optimization procedure calculates properties of the network under various environments, as described above, and identifies the specified environment in which the desired performance is achieved. The cells are cultured for a sufficient period of time and under conditions to allow the cells to evolve to the desired performance. That is, adaptive evolution of natural or engineered strains is carried out as guided by the general optimization methods or procedures.

Application at para. [0079] - [0082]

Therefore, the mere act of culturing a genetically altered cell is distinct from, and insufficient to teach or suggest the claimed evolution step. Instead, the genetic makeup as claimed is evolved to the desired optimal function after cultivation for a sufficient period of time because it does not necessarily manifest the optimal functions predicted by the *in silico* model. The cited references neither teach, suggest or motivate one skilled in the art to perform this claimed step.

For example, none of the cited references teach or suggest that "the resulting engineered cell may or may not display the optimal properties calculated ahead of time by the *in silico* methods using the iterative optimization procedure" described and claimed in the application.

Further, Edwards et al. does not perform the repeated steps of calculating optimal properties, altering a list of reactions until a desired optimal function is reached. Rather, Edwards et al. performs a robustness analysis of a constructed model that alters flux levels and calculates growth sensitivity to these altered fluxes. Edwards' *in silico* robustness analysis appears to purport the identification of three categories of gene products that are needed for growth on minimal medium. Edwards et al. makes no suggestion for repeatedly altering a list of

reactions to achieve a desired optimal function nor is there any suggestion that an evolutionary step is needed in order to achieve a desired optimal function determined by a repeated altering of a reaction list. Because Edwards et al. neither alters a list of reactions nor provides any indication that an evolutionary step is required to achieve a desired optimal function, Edwards et al. cannot provide the requisite teaching, suggestion or motivation to combine with the secondary references cited for culturing engineered cells. Similarly, for the reasons described previously with respect to Berry, neither Berry nor Varner et al. provide a teaching, suggestion or motivation to evolve cells because they are focused on induction and/or expression of engineered cells without any comparison to a model and without any evolution step. There is no description, expressed or implied, in Berry or Varner et al. that suggests evolving their engineered cells to achieve an optimal function that wasn't exhibited based on initial culture and expression.

With respect to the assertion that Edwards et al. describes that their *in silico* prediction has been experimentally confirmed, Edwards et al. simply point out that their results are consistent with an earlier report published by Fraenkel et al. Edwards et al. neither teaches nor suggests constructing a genetic makeup of a cell containing specific reaction networks, nor does Edwards et al. teach or suggest the claimed step of evolving such a makeup to allow the cells to evolve to the desired optimal function.

The Office further appears to assert that the standard for a reference to teach away requires a teaching that an invention "will not work under particular conditions." Applicants respectfully point out that an absolute requirement of inoperability is not the law. A reference teaches away when "a person of ordinary skill, upon reading the reference, would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the applicant." *In re Kahn*, Case No. 04-1616, slip op. at 19-20 (Fed. Cir. March 22, 2006) (*quoting In re Gurley*, 27 F.3d 551, 553 (Fed. Cir. 1994)).

The claimed invention expressly requires physiological process to be performed by cultivating to evolve a genetic makeup because the *in silico* calculated model may not yield the

desired result when it is actually constructed in a cell. In contrast, Edwards et al. expressly describe that their *in silico* model is sufficiently reliable to use as the basis for other *in silico* models with little experimental information. Hence, Edwards et al. discourages cultivating actual cells and evolving them to achieve their calculated, *in silico* result because Edwards et al. teaches that their models are sufficient and reliable. Accordingly, Edwards et al. teach away from the invention as claimed.

In light of the above remarks, Applicants maintain that Edwards et al, Varner et al. and Berry fail to provide the requisite teaching, suggestion or motivation to evolve a genetic makeup of a cell containing a biochemical reaction network specifying optimal properties to the desired optimal function as claimed by the invention. Withdrawal of this ground of rejection is respectfully requested.

CONCLUSION

In light of the Amendments and Remarks herein, Applicant submits that the claims are in condition for allowance and respectfully request a notice to this effect. Should the Examiner have any questions, he is invited to call the undersigned attorney.

Enclosed is check number 581458 in the amount of $620.00, which is the requisite fee of $395.00 for the Request for Continued Examination fee and $225.00 for the two-month Petition for Extension of Time fee. The Commissioner is hereby authorized to charge any other fees associated with the filing submitted herewith, or credit any overpayments to Deposit Account No. **07-1896**. A duplicate copy of the Transmittal Sheet is enclosed.

Respectfully submitted,

Date: April 12, 2006

Reg. No. 45,517

Per Lisa A. Haile, J.D., Ph.D.
Registration No.: 38,347
Telephone: (858) 677-1456
Facsimile: (858) 677-1465

DLA PIPER RUDNICK GRAY CARY US LLP
4365 Executive Drive, Suite 1100
San Diego, California 92121-2133
USPTO Customer Number 28213